

EVOLUTIONARY ANALYSIS OF POLLINARIA MORPHOLOGY OF SUBTRIBE AERIDINAE (ORCHIDACEAE)

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ABSTRACT

HIDAYAT, TOPIK; YUKAWA, TOMOHISA; ITO, MOTOMI. 2006. Evolutionary analysis of pollinaria morphology of subtribe *Aeridinae* (*Orchidaceae*). *Reinwardtia* 12(3): 223–235. — Pollinarium is one of the distinct synapomorphies of *Orchidaceae*. With using characters derived from the pollinarium, phylogenetic relationships among genera of subtribe *Aeridinae* was examined. Cladistic analysis showed that (1) Subtribe *Aeridinae* is monophyletic group. (2) Five of six groups constructed in the analysis are consistent with the groups recognized in previous molecular phylogenetic analyses. (3) The genera *Cleisostoma* and *Phalaenopsis* are non-monophyletic group. (4) Pollinarium morphology endorses monophyly *Trichoglottis* and *Phalaenopsis* alliances. (5) Although transformation of the stipe and viscidium shapes in the subtribe is subjected to parallelism, the results showed that these characters are much useful in determining relationships in the subtribe than those of pollinium.

Keywords: *Aeridinae*, *Orchidaceae*, Pollinarium, Cladistic

ABSTRAK

HIDAYAT, TOPIK; YUKAWA, TOMOHISA; ITO, MOTOMI. 2006. Analisis evolusi morfologi polinaria pada subtribe *Aeridinae* (*Orchidaceae*). *Reinwardtia* 12(3): 223–235. — Di dalam famili *Orchidaceae*, polinarium merupakan salah satu ciri pembeda dari famili tumbuhan *Angiospermae* lainnya, dan berperan dalam menyediakan karakter bagi penelitian-penelitian taksonomi dan filogenetika. Polinarium tersusun dari polinium dan organ-organ tambahan seperti kaudikel, stipe dan viscidium. Dengan menggunakan karakter morfologi dari polinarium, hubungan filogenetika diantara marga-marga yang tergolong ke dalam subtribe *Aeridinae* (famili *Orchidaceae*) telah diteliti. Analisis kladistik dengan melibatkan 90 jenis (50 marga) menunjukkan bahwa: (1) Subtribe *Aeridinae* adalah kelompok monofiletik. (2) Lima dari enam kelompok monofili yang dihasilkan sesuai dengan beberapa kelompok yang dihasilkan dari penelitian sebelumnya berdasarkan karakter molekuler. (3) Dua marga yaitu *Cleisostoma* dan *Phalaenopsis* bukan kelompok monofiletik. (4) Meskipun hasil penelitian menunjukkan bahwa karakter bentuk stipe dan viscidium mengalami evolusi paralel, kedua karakter ini secara taksonomi lebih berguna dibandingkan polinium.

Kata kunci: *Aeridinae*, *Orchidaceae*, Pollinarium, kladistik

INTRODUCTION

The pollinarium is defined as pollinia, a pollen mass, and accessory organs such as a caudicle, a stipe, and a viscidium. In *Orchidaceae*, this feature is an informative source both in taxonomy and phylogenetics (Freudenstein and Ramussen 1996) and has been considered to be less subject to parallelism than are other floral features (Chase 1987). In particular, pollinia characters have been used as a cardinal marker for classification systems such as Brown (1810) and Lindley (1826). Further, micromorphology of pollinia has often

given a great impact on the classification in various orchid groups (e.g., Ackerman and Williams 1980,1981; Schill and Wolter 1986; Zavada 1990; Freudenstein 1991,1994). Chase (1987), on the other hand, has used pollinarium structure in clarifying systematic problems in subtribe *Oncidiinae*.

The orchid subtribe *Aeridinae* is a large, diversified group with many taxonomic and phylogenetic problems among the members. The subtribe comprises 103 genera with approximately 1350 species, distributed throughout warm-temperate Asia to tropical Asia and Australia, and

the Pacific islands in the east. Two genera, *Taeniophyllum* and *Acampe*, extend the distribution as far west as tropical Africa. Vegetative parts of the subtribe are characterized by monopodial growth and highly developed velamentous layers of roots adapted to epiphytic life form. The pollinarium parts are characterized by two or four hard pollinia with a definite stipe and a viscidium. Several genera are further characterized by a column foot and a spurred lip.

Most systematic work in the subtribe has been concentrated at the generic level (e.g., Garay 1972; Christenson 1994). Several workers, however, have attempted to trace relationships at higher levels. Schlechter (1926) subdivided subtribe *Aeridinae* into two groups on the basis of presence or absence of the column foot. The usefulness of pollinia characters (number and aperture) to subdivide this group was outlined by Smith (1934) and was further elaborated by Holttum (1958), Senghas (1988), Seidenfaden (1988) and Dressler (1993), but they neglected information of the shape of stipe and viscidium.

Our recent study of molecular phylogenetics based on plastid *matK*, a maturase-coding gene, and internal transcribed spacers (ITS) of nuclear ribosomal DNA sequences recognized 14 monophyletic groups within the subtribe (Topik *et al.*, 2005). The result is inconsistent with previous classifications of the subtribe such as Schlechter (1926) and Senghas (1988). In addition, the result

indicated parallel evolution of characters such as pollinium number and presence or absence of column foot.

Although diversity in pollinarium structure in subtribe *Aeridinae* has been studied (Szlachetko 2003), no satisfactory conclusion existed in relation with the process leading to character evaluation, which would then be useful in inferring phylogenetic evidence. In this study, therefore, we re-examined phylogenetics implication of pollinarium morphology in subtribe *Aeridinae* with emphases on the structure of stipe and viscidium in more explicit cladistic approach. The cladistic analysis is useful for evaluating previous systems of classification in providing a concrete, explicit set of characters, and coding (Gravendeel 2000).

MATERIALS AND METHODS

A total of 90 species that represent 50 genera in subtribe *Aeridinae*, five genera of subtribe *Angraecinae*, and a single genus of subtribe *Aerangidinae* were examined. The two latter subtribes have been recognized as the sister group to subtribe *Aeridinae* on the basis of morphological characters (Dressler 1993) and macromolecular characters (Jarrell and Clegg 1995; Cameron *et al.*, 1999; Chase 2005; van den Berg *et al.*, 2005). The plant materials are shown in Table 1.

Table 1. Plant materials examined in this study.

Taxon	Sources	Voucher
1	2	3
Tribe <i>Vandaeae</i>		
Subtribe <i>Angraecinae</i>	TBG	NA
<i>Angraecum germinyanum</i> Hort. Sand. ex Hook.f.	TBG	NA
<i>Aeranthes orthopoda</i> Toill.-Gen., Ursch & Bosser	TBG	TBG133855
<i>Cribbia confusa</i> P.J. Cribb	TBG	TBG140595
<i>Jumellea sagittata</i> H.Perrier	TBG	TBG134582
<i>Calypstrochilum christyanum</i> (Rchb.f.) Summerh.		
Subtribe <i>Aerangidinae</i>	TBG	NA
<i>Podangis dactyloceras</i> Schltr.		
Subtribe <i>Aeridinae</i>	TBG	B200107222
<i>Abdominea minimiflora</i> J.J.Sm.	TBG	TBG142425
<i>Adenoncos parviflora</i> Ridl.	TBG	TBG144183
<i>Aerides flabellata</i> Rolfe ex Downie	TBG	TBG85031
<i>Aerides houlettianum</i> Rchb.f.	TBG	TBG118176
<i>Aerides krabiensis</i> Seidenfaden	TBG	TBG118480
<i>Aerides odorata</i> Lour.	TBG	TBG180168
<i>Acampe ochracea</i> Hochr.	TBG	TBG56086
<i>Acampe rigida</i> (Buch.-Ham. ex Sm.) P.F. Hunt	TBG	TBG123790
<i>Amesiella monticola</i> J.E. Cootes & D.P. Banks	TBG	TBG133686
<i>Ascocentrum ampullaceum</i> Schltr.	TBG	NA
<i>Ascocentrum christensonianum</i> J.R. Haager	TBG	TBG145826
<i>Ascocentrum garayi</i> Christenson	TBG	TBG100228
<i>Ascocentrum pusillum</i> Averyanov	TBG	TBG130213

Taxon	Sources	Voucher
1	2	3
<i>Ascochilus siamensis</i> Ridl.	TBG	TBG144146
<i>Brachypeza zamboangensis</i> (Ames) Garay	TBG	TBG145835
<i>Ceratocentron fesseli</i> Senghas	TBG	TBG133203
<i>Ceratochilus biglandulosus</i> Blume	TBG	TBG144188
<i>Chiloschista parishii</i> Seidenfaden	THAI (Chiang Mai)	NA
<i>Christensonia vietnamica</i> J.R. Haager	TBG	TBG118224
<i>Cleisomeria lanatum</i> Lindl. ex G.Don	TBG	TBG126617
<i>Cleisostoma arietinum</i> (Rchb.f.) Garay	TBG	TBG84208 or 118430
<i>Cleisostoma</i> aff. <i>gjellerupii</i> (J.J.Sm.) Garay	TBG	Cult. K. Tsukahara
<i>Cleisostoma javanicum</i> (Bl.) Garay	TBG	NA
<i>Cleisostoma weberi</i> (Ames) Garay	TBG	TBG128820
<i>Cleisostoma uraiense</i> (Hayata) Garay & H.R. Sweet	TBG	NA
<i>Doritis pulcherrima</i> Lindl.	TBG	TBG118344
<i>Dyakia hendersoniana</i> (Rchb.f.) Christenson	TBG	TBG133581
<i>Gastrochilus calceolaris</i> D.Don	TBG	TBG142434
<i>Gastrochilus obliquus</i> Kuntze	TBG	NA
<i>Grosourdyia callifera</i> Seidenf.	THAI (Chiang Mai)	TBG145840
<i>Haraella retrocalla</i> Kudo	TBG	TBG133078
<i>Holcoglossum subulifolium</i> (Rchb.f.) Christenson	TBG	TBG141082
<i>Hygrochilus parishii</i> Pfitzer	TBG	TBG118479
<i>Lesliea mirabilis</i> Seidenf.	THAI	TBG145844
<i>Luisia amesiana</i> Rolfe	TBG	TBG128939
<i>Malleola witteana</i> J.J.Sm. & Schltr. ex Schltr.	TBG	TBG140471
<i>Micropera philippinensis</i> Lindl.	TBG	NA
<i>Micropera</i> spp	TBG	TBG118437
<i>Micropera</i> spp	TBG	TBG141027
<i>Microsaccus griffithii</i> (Par. & Rchb.f.) Seidenf.	TBG	TBG129769
<i>Neofinetia falcata</i> Hu.	TBG	TBG140668
<i>Omoea philippinensis</i> Ames	TBG	TBG133261
<i>Paraphalaenopsis laycockii</i> (M.R. Hend.) A.D. Hawkes	TBG	TBG134851
<i>Pelatantheria ctenoglossum</i> Ridl.	TBG	TBG118382
<i>Phalaenopsis amabilis</i> Blume	TBG	TBG145847
<i>Phalaenopsis appendiculata</i> Carr	TBG	NA
<i>Phalaenopsis chibae</i> T. Yukawa	TBG	TBG115846
<i>Phalaenopsis fasciata</i> Rchb.f.	TBG	TBG145726
<i>Phalaenopsis lowii</i> Rchb.f.	TBG	TBG144316
<i>Pomatocalpa kunstleri</i> J.J.Sm.	TBG	TBG145833
<i>Pomatocalpa spicatum</i> Breda	THAI (Bangkok)	NA
<i>Pteroceras fragrans</i> (Ridl.) Garay	TBG	NA
<i>Pteroceras pallidum</i> (Bl.) Holttum	TBG	TBG140670
<i>Pteroceras semiteretifolium</i> H.A. Pedersen	TBG	TBG133232
<i>Renanthera bella</i> J.J. Wood	TBG	TBG134821
<i>Renanthera angustifolia</i> Hook.f.	TBG	TBG124337
<i>Renanthera</i> spp	TBG	TBG140551
<i>Rhyncostylis coelestis</i> Rchb.f.	TBG	TBG100261
<i>Rhyncostylis gigantea</i> (Lindl.) Ridl.	TBG	TBG78872
<i>Rhyncostylis retusa</i> (L.) Blume	TBG	TBG118423
<i>Robiquetia cerina</i> (Rchb.f.) Garay	TBG	TBG126665
<i>Robiquetia bertholdii</i> Schltr.	TBG	TBG125177
<i>Saccolabium pusillum</i> Bl.	TBG	TBG145481
<i>Sarcoglyphis comberi</i> (J.J. Wood) J.J. Wood	TBG	TBG144127
<i>Schoenorchis fragrans</i> (Parish & Rchb.f.) Seidenf. & Smitin.	TBG	NA
<i>Schoenorchis paniculata</i> Bl.	TBG	TBG140487
<i>Schoenorchis secundiflora</i> J.J.Sm.	MAL (Cameron Highland)	NA
<i>Sedirea japonica</i> (L. Linden & Rchb.f.) Garay & HR. Sweet	TBG	TBG145832
<i>Seidenfadenia mitrata</i> (Rchb.f.) Garay	TBG	TBG141188
<i>Staurochilus ionosma</i> Schltr.	TBG	TBG130159
<i>Stereochilus</i> aff. <i>dalatensis</i> (Guill.) Garay	TBG	TBG127489
<i>Thrixspermum centipeda</i> Lour	TBG	TBG118459
<i>Trichoglottis latisejala</i> Ames var. <i>tricarinata</i> T. Hashimoto	TBG	TBG79675
<i>Trichoglottis philippinensis</i> Lindley	TBG	NA
<i>Trichoglottis pusilla</i> Rchb.f.	TBG	TBG132859
<i>Trichoglottis wenzellii</i> Ames	TBG	TBG130161
<i>Trudelia cristata</i> (Lindl.) Senghas	TBG	NA
<i>Trudelia pumila</i> (Hook.f.) Senghas	TBG	TBG118899
<i>Tuberolabium escritorii</i> (Ames) Garay	TBG	TBG141159
<i>Tuberolabium odoratissimum</i> (J.J.Sm.) Garay	TBG	NA
<i>Vanda roeblingiana</i> Rolfe	TBG	TBG118900
<i>Vanda tricolor</i> Lindley	TBG	NA
<i>Ventricularia tenuicaulis</i> (Hook.f.) Garay	THAI (Chiang Mai)	TBG145846

The materials were collected from Tsukuba Botanical Garden-Japan (TBG), Thailand (THAI) and Malaysia (MAL). NA= Not available

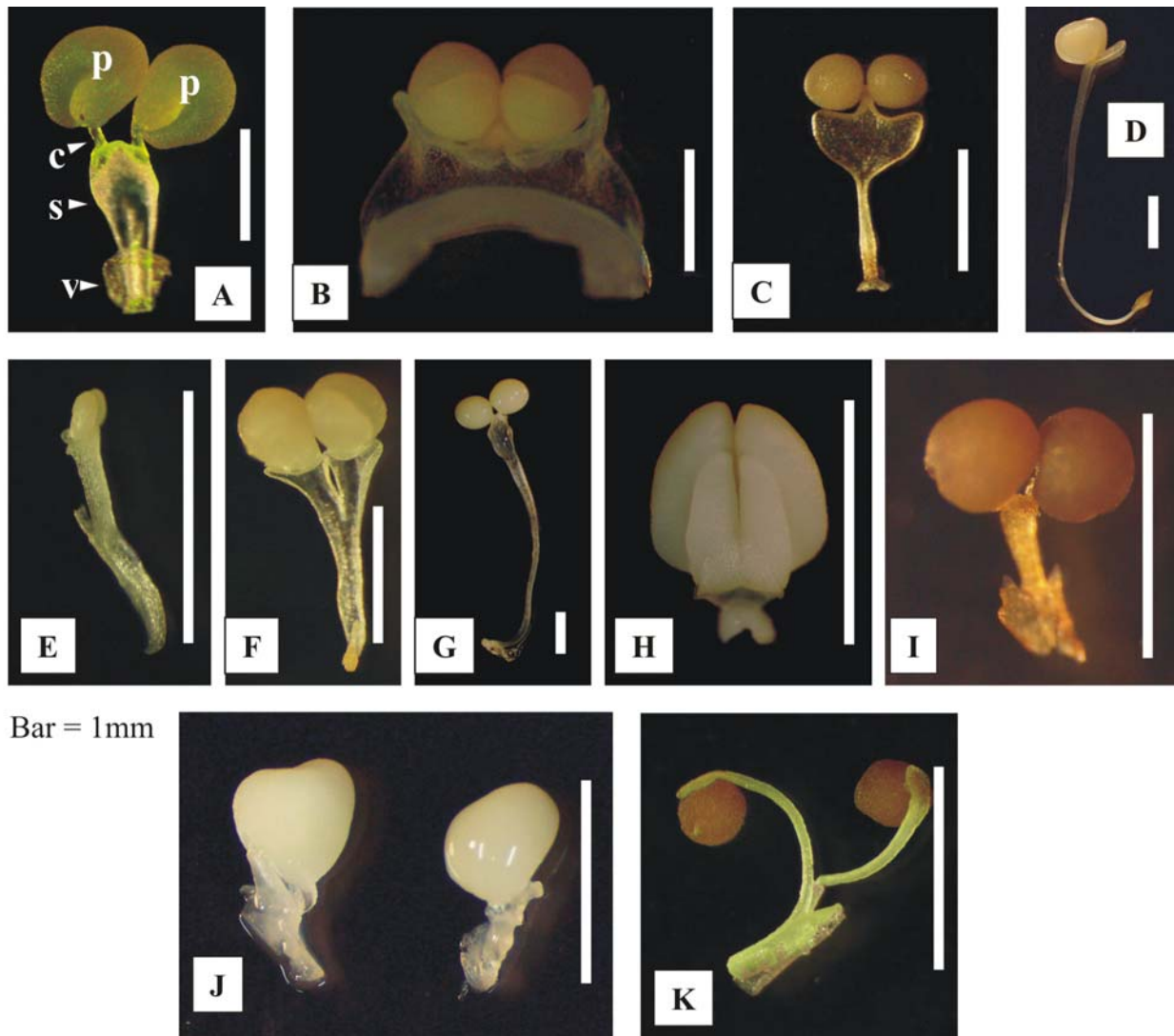


Fig. 1. Pollinarium diversity of some represented genera in subtribe *Aeridinae* (A-H) and the *Angraecoids* group (I-K). A. *Brachypeza zamboangensis*. B. *Pelatantheria ctenoglossum*. C. *Malleola witteana*. D. *Phalaenopsis lowii*. E. *Schoenorchis fragrans*. F. *Cleisomeria lanatum*. G. *Micropera philippinensis*. H. *Thrixspernum centipeda*. I. *Calyptrochilum christyanum*. J. *Angraecum germinyanum*. K. *Podangis dactyloceras*. p= pollinium, c= caudicle, s= stipe, v= viscidium.

For pollinarium preparation, the methods described in Chase (1987) were used with several modifications. Pollinarium morphology was observed only from living materials due to the fact that: (1) the caudicle is easy to dry and dissolve in alcohol and permits the pollinia to fall away, (2) the viscidium is easy to change its shape in alcohol, and (3) intact pollinaria are easy to remove. Pollinaria were observed using a light microscope and a stereo-dissecting microscope, illustrated on a drawing book, photographed using a stereo - dissecting microscope, and stored in 70% ethanol.

The cladistic analysis was conducted with PAUP version 4.0 v 10 (Swofford 1998). Homogeneous genera were represented by a single

species only, whereas larger, more variable genera were represented by several species. In total, 59 taxa were used in the analysis. All character states were equally weighted and unordered (Fitch 1971). The data set was analyzed by the heuristic search method with tree bisection-reconnection (TBR) branch swapping and the MULTREES option on, saving all most parsimonious trees (MPTs). Evaluation of internal support of clades was conducted by the bootstrap analysis (Felsenstein 1985) with 1,000 replicates, simple stepwise addition, TBR branch swapping, and the MULTREES option off. The number of steps, consistency indices (CI), and retention indices (RI) were calculated on one of the MPTs using the TREE SCORES command in PAUP*.

RESULTS

Characters

As found in some orchid groups, e.g., subtribe *Oncidiinae* (Chase 1987), a substantial diversity of the pollinia (p); the caudicle (c), thread connecting between the pollinia and the apex of the stipe; a cellular stipe (s), thread connecting between the caudicle and viscidium; and a sticky viscidium (v), viscid plate adhering to the pollen vector (Fig. 1-A) was observed in subtribe *Aeridinae*. In this study, we found some typical features of pollinarium structure in subtribe *Aeridinae*; the stipe is flattened of variable width and length and the pollinia orientation when four is typically superposed, the pairs of pollinia are stacked one on another. Characterization and scoring were made for each character state and were summarized in Table 2.

Pollinium- In their molecular phylogenetic study, Topik *et al.*, (2005) suggested the ancestral condition of two pollinia in subtribe *Aeridinae*. Most genera studied have either compressed or globular pollinia. Only a single genus, *Thrixspermum* (Fig. 1-H), has peculiar pollinia shape, triangular. *Phalaenopsis* (Fig. 1-D), *Doritis*, and *Lesliea* have pollinia that ventrally attach to the stipe.

Stipe- Subtribes *Angraecinae* and *Aerangidinae*, the outgroups of our study, have diversified shape of stipe. Predominantly, they have a separate double stipe, of which each part is either attached to a separate viscidium (e.g., *Angraecum*; Fig. 1-J) or share a common viscidium (e.g., *Podangis*; Fig. 1-K), but a few have a single stipe (e.g., *Calyptrochilum*; Fig. 1-I). In contrast, the stipe of subtribe *Aeridinae* is always single with great diversity in shape. For example, *Cleisomeria* (Fig. 1-F) has a “Y”-like stipe whereas *Malleola* (Fig. 1-C) has a “wing”-like stipe.

Length of stipe within subtribe *Aeridinae* varies very much. We scored very long stipe (> seven times longer than the diameter of pollinia) (e.g., *Micropera*; Fig. 1-G) to be the derived state due to the fact that no outgroup taxa demonstrates this state.

The stipe shape varies from strap-like (e.g., *Brachypeza*; Fig. 1-A), linier (e.g., *Micropera*; Fig. 1-G), to very broad (e.g., *Pelatantheria*; Fig. 1-B). Chase (1987) used this character as a major feature to infer the affinity of subtribe *Oncidiinae*.

Caudicle- The caudicle facilitates pollinia to

divide into each pollinium (Rasmussen 1986). Freudenstein and Rasmussen (1999) showed that the caudicle is one of important states for orchid relationships. The prominent state seems to be stable in a few genera examined such as *Brachypeza* (Fig. 1-A).

Table 2. Pollinarium characters and polarization characters.

No.	Characters	Character states
1.	Number and aperture of pollinium	0= two-cleft or porate 1= two-entire 2= four
2	Shape of pollinium	0= compress~globular 1= triangular
3.	Attachment of pollinium	0= apical 1= ventral
4.	Number of stipe	0= double 1= single
5.	Length of stipe	0= short~long (\leq seven times of diameter pollinium) 1= very long ($>$ seven times of diameter pollinia)
6.	Shape of stipe	0= narrow 1= strap 2= rectangular 3= linier 4= broadening at apex (“wing”-like) 5= broadening toward the apex 6= “Y”-like
7.	Basal caudicles	0= not prominent 1= prominent
8.	Shape of viscidium	0= triangular 1= quadrangular 2= more and less oval 3= surfboard-like 4= irregular
9.	Broad viscidium	0= absent 1= present

Viscidium- The viscidium plays role in attaching the pollinia to an insect allowing the pollinia to be carried to another flower. Fruedenstein and Rasmussen (1999) recognized detachable group of viscidium (sticky pad-like structure), against to diffuse group, a much less elaborate structure, looking like a glue. In subtribe *Aeridinae*, we recognized five states: triangular, quadrangular, oval, surfboard-like and irregular. In addition, a

very broad viscidium was found in several genera such as *Schoenorchis* (Fig. 1-E).

Tree topology

102,5 MPTs, with length= 25 steps, CI= 0.80, and RI= 0.89 were generated from the analysis of nine characters and 59 taxa (Table 3). The tree shown in Fig.2 is strict consensus of the 1025 MPTs. Although the tree is not well resolved as deduced by the low bootstrap percentages (BP<50) in most clades, six groups are recognized. All the ingroup taxa share an apomorphic states, a single stipe. Group I includes *Ascochilus*, *Brachypeza*, *Grosourdyia*, and *Pteroceras*, which is defined by the prominent basal caudicle, strap-like stipe, and two pollinia.

Phalaenopsis, *Doritis*, and *Lesliea* make up group II, characterized by the ventral attachment of pollinia to the apex of stipe and broadening stipe toward the apex. Group III has a quadrangular viscidium, two pollinia, and a strap-like stipe, comprising *Robiquetia*, *Omoea*, *Maleolla*, *Aerides*, *Holcoglossum*, *Ascocentrum*, *Christensonia*, *Neofinetia*, *Rhyncostylis*, *Vanda*, and *Trudelia*.

Pollinarium morphology in all of the members of group IV is defined by the linear, very long stipe. Oval viscidium characterizes *Micropera*, *Sarcoglyphis*, and *Dyakia*, whereas *Seidenfadenia* has quadrangular viscidium.

Placement of three sampled species of *Cleisostoma*, namely, *C. weberi*, *C. arietinum*, and *C. javanicum*, suggests a close relationship to *Schoenorchis* and *Pelatantheria* (group V), because they all have a very broad viscidium; viscidium shape is either surfbroad-like or irregular. Group VI comprises *Trichoglottis*, *Staurochilus*, *Ceratochilus*, and *Ventricularia*, all of which share a quadrangular viscidium and four pollinia.

DISCUSSION

Characteristics of morphological data

In cladistic analyses of morphological data, support values such as bootstrapping (Felsenstein, 1985), Bremer support (Bremer, 1988), and jackknifing (Farris, 1996) are often much lower than those in cladistic analyses of molecular data. Many groups are only supported by a few characters, simply because the ratio of characters to taxa is small and many of these characters are stem, a stout column, and a broad epichile of the lip (Christenson 1987, 1994). An exception is

homoplasious (Freudenstein and Rasmussen 1999). In these cases, support indices become less important to evaluate the robustness of each clade. In this study only group V had bootstrap percentages more than 50 (Fig. 2).

Intergeneric relationships

Pollinarium morphology has provided valuable source of characters to evaluate hypothesis of relationships within subtribe *Aeridinae*. Although the number of character found here is somewhat meager (only nine characters), pollinarium analysis has added another features from which such systematic information may be derived. Smith (1934) noticed that the shape of stipes and viscidium could be useful characters in determining major systematic groups in subtribe *Aeridinae*.

In accordance with the results of molecular phylogenetic analysis based on *matK* and ITS regions (Topik *et al.*, 2005), the results of pollinarium analysis (Fig. 2) supported the monophyly of subtribe *Aeridinae*. Moreover, five of the six groups recognized in the pollinarium analysis agree with groups resulted in molecular phylogenetic analysis (Topik *et al.*, 2005). Group I, consisting of *Brachypeza*, *Ascochilus*, *Pteroceras*, and *Grosourdyia*, is considerably appeared to be a monophyletic group because this group has a synapomorphic state, the prominent basal caudicle.

Several genera recognized as *Phalaenopsis* alliance in molecular analyses (Topik *et al.*, 2005) have pollinia attached ventrally to the broadening stipe toward the apex. Moreover, we confirmed that genus *Phalaenopsis* is non-monophyletic group as has been reported in previous study (Topik *et al.*, 2005). As depicted in Fig. 2, within this group, species of *Phalaenopsis* is split according to their pollinium number: one is with two pollinia and the other is with four pollinia. According recent study of molecular phylogenetic of *Phalaenopsis* and allied genera (see detail in Yukawa *et al.*, 2005), the latter has been transferred to the genus *Doritis*. Most members of the *Aerides* alliance examined here: *Aerides*, *Holcoglossum*, *Ascocentrum*, *Christensonia*, *Neofinetia*, *Rhyncostylis*, *Vanda*, and *Trudelia* merged in group III, by the quadrangular viscidium and long stipe (\leq seven times longer than the diameter of pollinium). Further, some of the traditional character states have defined this group. These character states include a long leafy *Seidenfadenia*, which is embedded within group IV due to having very long stipe.

Table 3. Data matrix for the cladistic analysis. Character numbers as in Table 2.

No	Taxon	1	2	3	4	5	6	7	8	9
1	<i>Abdominea miniflora</i>	2	0	0	1	0	1	0	2	0
2	<i>Adenoncos parviflora</i>	2	0	0	1	0	1	0	2	0
3	<i>Aerides odorata</i>	0	0	0	1	0	1	0	1	0
4	<i>Acampe ochracea</i>	2	0	0	1	0	1	0	2	0
5	<i>Amesiella monticola</i>	0	0	0	1	0	1	0	2	0
6	<i>Angraecum germinyanum</i>	0	0	0	0	0	0	0	0	0
7	<i>Armadorum sullingii</i>	0	0	0	1	0	1	0	0	0
8	<i>Ascocentrum ampullaceum</i>	0	0	0	1	0	1	0	1	0
9	<i>Ascochilus siamensis</i>	0	0	0	1	0	1	1	0	0
10	<i>Brachypeza zamboangensis</i>	0	0	0	1	0	1	1	0	0
11	<i>Ceratocentron fessellii</i>	1	0	0	1	0	1	0	2	0
12	<i>Ceratochilus biglandulosus</i>	2	0	0	1	0	1	0	1	0
13	<i>Chiloschista parishii</i>	2	0	0	1	0	1	0	2	0
14	<i>Christensonia vietnamica</i>	0	0	0	1	0	1	0	1	0
15	<i>Cleisomeria lanatum</i>	2	0	0	1	0	6	0	2	0
16	<i>Cleisostoma arietinum</i>	2	0	0	1	0	2	0	3	1
17	<i>Cleisostoma aff. gjellerupii</i>	2	0	0	1	0	1	0	2	0
18	<i>Cleisostoma javanicum</i>	2	0	0	1	0	2	0	3	1
19	<i>Cleisostoma weberi</i>	2	0	0	1	0	2	0	3	1
20	<i>Cleisostoma uraiense</i>	2	0	0	1	0	1	0	2	0
21	<i>Doritis pulcherrima</i>	2	0	1	1	1	5	0	0	0
22	<i>Dyakia hendersoniana</i>	0	0	0	1	1	3	0	2	0
23	<i>Gastrochilus calceolaris</i>	0	0	0	1	0	1	0	0	0
24	<i>Grosourdyia callifera</i>	1	0	0	1	0	1	1	0	0
25	<i>Haraella retrocalla</i>	0	0	0	1	0	1	0	2	0
26	<i>Holcoglossum subulifolium</i>	0	0	0	1	0	1	0	1	0
27	<i>Hygrochilus parishii</i>	2	0	0	1	0	1	0	2	0
28	<i>Lesliea mirabilis</i>	2	0	1	1	0	5	0	0	0
29	<i>Luisia amesiana</i>	0	0	0	1	0	1	0	0	0
30	<i>Malleola witteana</i>	0	0	0	1	0	4	0	1	0
31	<i>Micropera philippinensis</i>	2	0	0	1	1	3	0	2	0
32	<i>Microsaccus griffithii</i>	2	0	0	1	0	1	0	2	0
33	<i>Neofinetia falcata</i>	0	0	0	1	0	1	0	1	0
34	<i>Omoea philippinensis</i>	1	0	0	1	0	1	0	1	0
35	<i>Paraphalaenopsis laycockii</i>	0	0	1	1	0	1	0	0	0
36	<i>Pelatantheria ctenoglossum</i>	2	0	0	1	0	2	0	4	1
37	<i>Phalaenopsis amabilis</i>	1	0	1	1	0	5	0	0	0
38	<i>Phalaenopsis appendiculata</i>	2	0	1	1	0	5	0	0	0
39	<i>Phalaenopsis fasciata</i>	0	0	1	1	0	5	0	0	0
40	<i>Phalaenopsis chibae</i>	2	0	1	1	0	5	0	0	0
41	<i>Phalaenopsis lowii</i>	2	0	1	1	1	5	0	0	0
42	<i>Pomatocalpa kunstleri</i>	2	0	0	1	0	1	0	2	0
43	<i>Pteroceras semiteretifolium</i>	0	0	1	1	0	1	1	0	0
44	<i>Renanthera angustifolia</i>	2	0	0	1	0	1	0	0	0
45	<i>Rhyncostylis retusa</i>	0	0	0	1	0	1	0	1	0
46	<i>Robiquetia bertholdii</i>	0	0	0	1	0	1	0	1	0
47	<i>Saccolabium pusillum</i>	1	0	0	1	0	1	0	2	0
48	<i>Sarcoglyphis comberi</i>	2	0	0	1	1	3	0	2	0
49	<i>Schoenorchis fragrans</i>	2	0	0	1	0	1	0	3	1
50	<i>Sedirea japonica</i>	0	0	0	1	0	1	0	0	0
51	<i>Seidenfadenia mitrata</i>	0	0	0	1	1	3	0	2	0
52	<i>Staurochilus ionosma</i>	2	0	0	1	0	1	0	1	0
53	<i>Stereochilus aff. dalatensis</i>	2	0	0	1	0	5	0	2	0
54	<i>Thrixspermum centipeda</i>	2	1	0	1	0	1	0	2	0
55	<i>Trichoglottis latisejala</i>	2	0	0	1	0	1	0	1	0
56	<i>Trudelia pumila</i>	0	0	0	1	0	1	0	1	0
57	<i>Tuberolabium escritorii</i>	1	0	0	1	0	1	0	2	0
58	<i>Vanda tricolor</i>	0	0	0	1	0	1	0	1	0
59	<i>Ventricularia tenuicaulis</i>	2	0	0	1	0	4	0	1	0

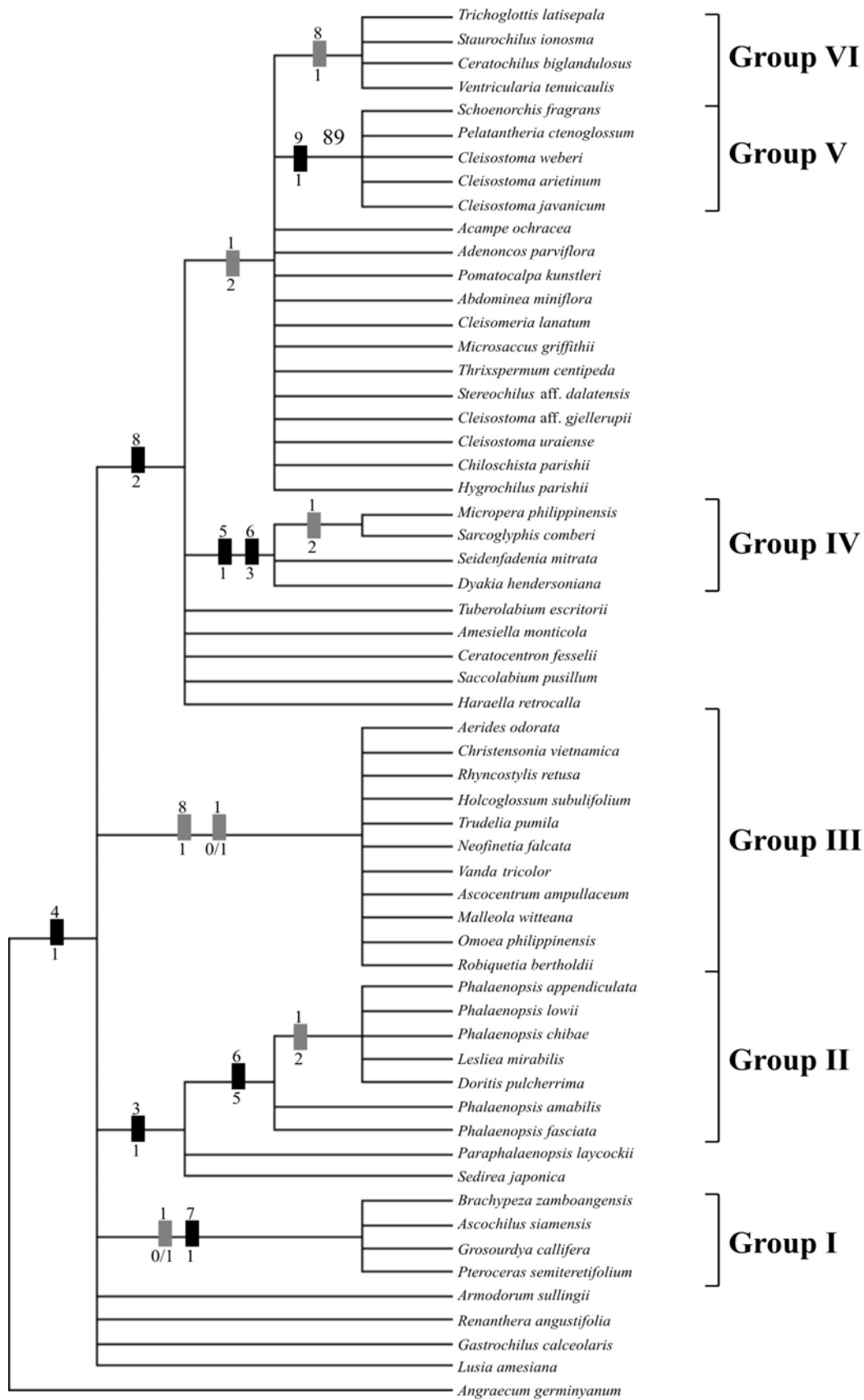


Fig. 2. Strict consensus tree of 1025 MPTs (Length= 25 steps, CI= 0.80, RI= 0.89) derived from pollinarium morphology. Bootstrap percentage of >50 is given about branch. Black bars are synapomorphies transformed only once and gray bars are synapomorphies transformed more than once. Character numbers are above bars and states are indicated below.

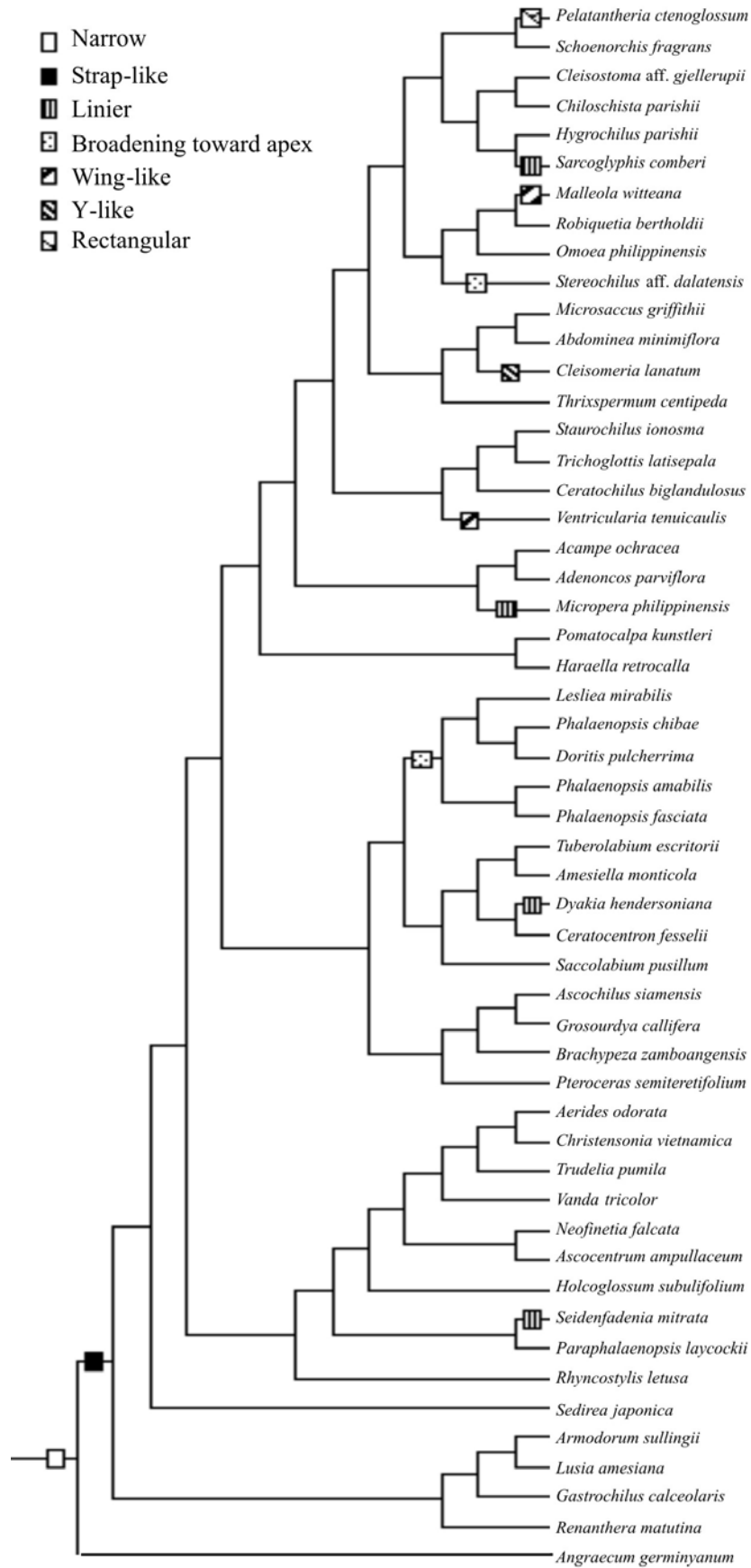


Fig. 3. Stipe reconstruction mapped on one molecular tree (Topik *et al.*, 2005).

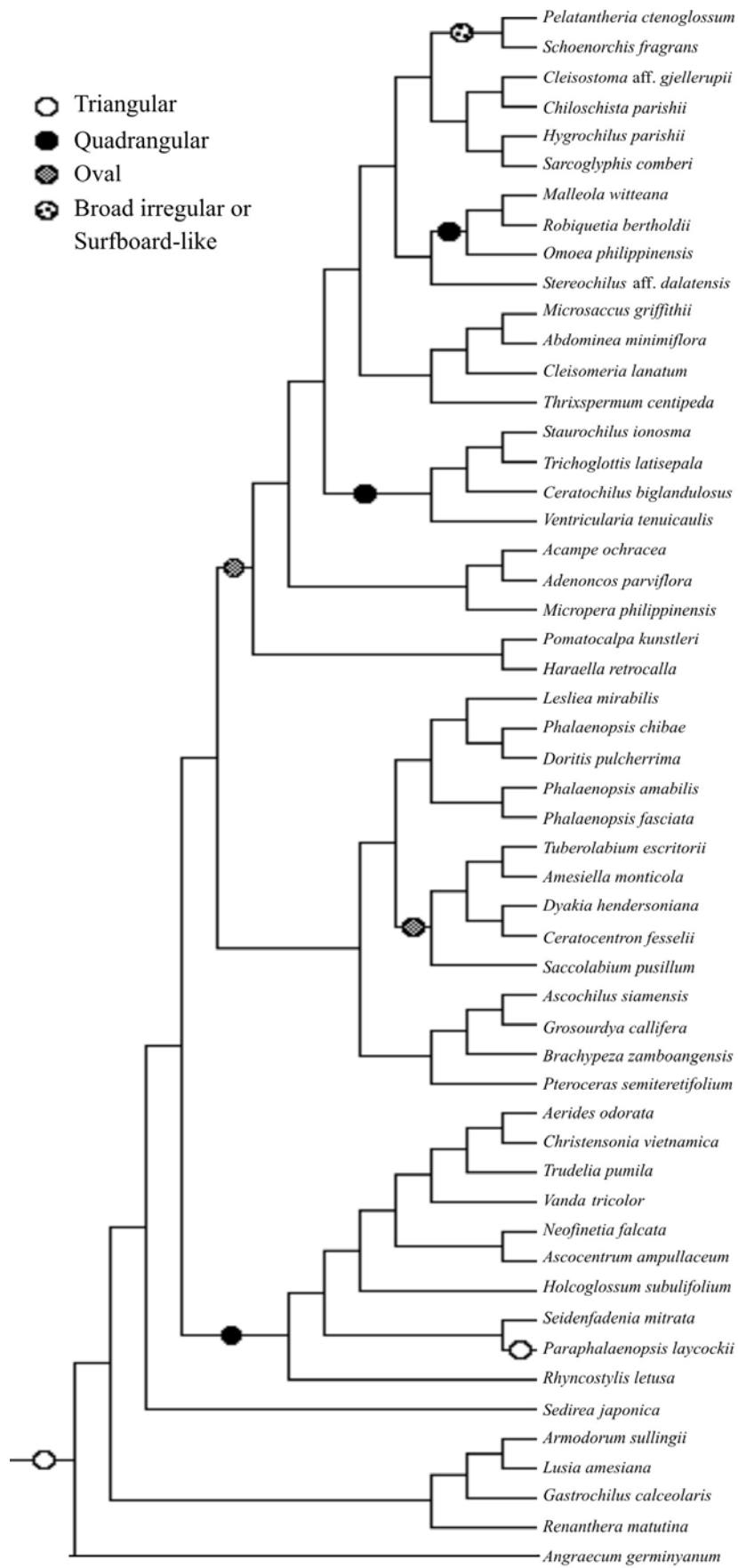


Fig. 4. Viscidium reconstruction mapped on one molecular tree (Topik *et al.*, 2005).

The grouping of *Dyakia*, *Sarcoglyphis*, *Seidenfadenia*, and *Micropera* (group IV) provides insight into affinities among them. They are united by very long stipe. Molecular phylogenetic analyses showed that these four genera have polyphyletic relationships (Topik *et al.*, 2005).

Heterogeneity of a large, complex genus *Cleisostoma* recognized by molecular tree (Topik *et al.*, 2005) has been confirmed in the present study. Three sampled species of *Cleisostoma* (*C. weberi*, *C. javanicum*, and *C. arietinum*), *Pelatantheria* and *Schoenorchis* are united (group V) by a unique synapomorphic state, broad viscidium with either irregular or surfboard-like in shape. The remaining two sampled species of *Cleisostoma* (*C. gjellerupii* and *C. uraiense*) are scattered in the tree.

As found in our molecular analysis (Topik *et al.*, 2005), *Trichoglottis* alliance contains *Trichoglottis*, *Ceratochilus*, *Staurochilus*, and *Ventricularia*, and that this group also share a morphological character, raising tongue across the spur from the column base to the spur base (Seidenfaden, 1988). A pollinarium character state, quadrangular viscidium, is further support this grouping.

Evolution of stipe and viscidium

The significance of stipe and viscidium characters has been received much attention in orchid taxonomy for a long time (e.g., Williams 1970a, 1970b, 1972; Chase 1987). Since this study showed that the shape of stipe and viscidium varies greatly in subtribe *Aeridinae*, we tested the evolutionary trend of these characters. Using MacClade 3.05 under accelerated character transformation (ACCTRAN) optimization, we thus mapped the character states of stipe and viscidium shape onto one tree derived from molecular data (Topik *et al.*, 2005).

As shown in Fig. 3, seven states of stipe shape were recognized in the subtribe in which the narrow stipe is the ancestral state. The strap-like stipe that appeared once is likely an intermediate form from which other derived states are evolved. While the linear stipe likely evolved four times, the wing-like and broadening toward the apex stipes probably appeared two times. The rectangular and "Y"-like stipes occurred once. The viscidium shape, on the other hand, comprises four states in which the triangular shape represents the plesiomorphic state (Fig. 4). The quadrangular and oval viscidiums were shown to evolve three times and twice respectively, whereas the broad irregular or surfboard-like viscidium appeared once. On the other hand, reversal occurred once from the quadrangular to the triangular viscidiums. These facts indicated that changes of states both in stipe

and viscidium shapes occurred repeatedly in subtribe *Aeridinae* as many other similar evolutionary events are found in *Orchidaceae*.

Pollinarium versus pollinators

Relationships between the diversity of pollinarium morphology in subtribe *Aeridinae* and their pollinators still remain unresolved. Pollinia characters are not likely to be correlated with particular classes of pollinators as the hard pollinium cannot be used as food by pollinators. Besides, pollinia are enclosed within an anther cap so that not visible by pollinators; color and shape of pollinia are not related to pollinators preferences. It seems logical to suppose that there is no direct relationship between pollinium characters and pollinators in subtribe *Aeridinae*. However, there is an assumption that the diversity of pollinarium structure could be stimulated under the influence of evolutionary interactions between flowers and pollinators (Cozzolino *et al.*, 2001).

It is well known that morphological characters of stipe and viscidium are directly related with functions in pollination (Dressler 1981, 1993). The stipe plays a role in determining efficiency of pollination in which, if long, the pollinia are relatively easy to reach stigma to initiate pollination. Viscidium is functioned in transportation of the pollinia from one flower to another via a pollinator as "vehicle" to which it is attached. Consequently, its shape (and size as well) seems likely to be a factor in mechanism of attachment to the pollinator's body either the head or the trunk. Accordingly, with having of a broad viscidium enable plant prone to attach to the pollinator's body and lead to an increased probability of pollination, or the reverse. The diversity of stipe and viscidium found in this study, therefore, indicate diversity of evolutionary interactions between flowers and pollinators in the subtribe, which would then promote diversity of pollinarium structure as a whole.

CONCLUSION

The reconstruction of cladogram based on pollinarium morphology has substantially illuminated systematic usefulness of this feature in subtribe *Aeridinae*. The results supported the monophyly of subtribe *Aeridinae*. Five of six groups revealed in this analysis fit with groups recognized in the molecular data of subtribe *Aeridinae*. Pollinarium morphology endorses monophyly of *Trichoglottis* and *Phalaenopsis* alliances and supports non-monophyly of

Cleisostoma and *Phalaenopsis* indicated by molecular characters. Although transformation of the stipe and viscidium shapes in the subtribe is subjected to parallelism, the results showed that these characters are much useful in determining relationships in the subtribe than those of pollinium (number and aperture) as indicated by our previous molecular analyses (Topik *et al.*, 2005). Further cladistic analysis through rigorously examining more characters of pollinarium morphology and utilizing more taxon intensities will improve the results and reveal more reliable hypothesis on relationships of subtribe *Aeridinae*. Outputs from pollination biology of the group are much needed to understand the character evolution of the pollinarium.

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